

THE MICHIGAN PHYSIDAE REVISITED: A POPULATION GENETIC SURVEY

Robert T. Dillon, Jr.¹ & Amy R. Wethington²

ABSTRACT

We report an analysis of gene frequencies at 7 polymorphic allozyme-encoding loci in 16 populations of physid snails collected from Michigan, surveyed as a step toward integrating Te's (1978) influential classification of the Physidae with a more comprehensive system based on genetic interrelationships and breeding data. Analysis of a genetic distance matrix revealed three groups – two populations of *Aplexa hypnorum* together, five populations of *Physa acuta* together, and nine populations of *P. gyrina*, *P. sayii*, and *P. parkeri* combined. Allozyme divergence among the populations of this last cluster, referred to as the “*gyrina* group,” was comparable to that seen among the five populations of the well-characterized *P. acuta* cluster, which breeding experiments have demonstrated biologically conspecific. These results suggest that Michigan populations assigned to *P. gyrina*, *P. sayii*, and *P. parkeri* may comprise a single biological species, the globose and often shouldered shell morphology of the latter resulting from local and perhaps phenotypically plastic responses to lacustrine environments. The 14 “taxonomic units” from Michigan that Te included in his analysis may represent as few as four biological species. A reduction in nominal higher levels of classification within the Physidae is called for.

Key words: Gastropoda, Pulmonata, *Physella*, allozyme polymorphism, protein electrophoresis.

INTRODUCTION

The freshwater pulmonate family Physidae includes some of the more common and widespread gastropod species on earth (Burch, 1989; Dillon, 2000; Dillon et al., 2002). In North America, the most influential classification of the family is currently that of George A. Te (1978, 1980). Te's analysis, based on 71 characters scored primarily from the shell and reproductive anatomy, suggested that the 85 taxonomic units he recognized might be divided into four genera: *Aplexa*, *Stenophysa*, *Physa* and *Physella*, the last genus with three subgenera (*Petrophysa*, *Costatella*, and *Physella* s.s.). This classification was adopted by Burch for his “North American Freshwater Snails” (Burch, 1989), and subsequently by Brown (1991), Turgeon et al. (1998), and many others.

A wealth of data regarding genetic relationships among the North American physids has accumulated in the 25 years since Te proposed his classification. Reports have been published detailing gene frequencies at allozyme-encoding loci among a variety of nominal species

(Buth & Salloway, 1983; Liu, 1993; Dillon & Wethington, 1995; Jarne et al., 2000). More recently, data have become available on DNA sequence divergence (Remigio et al., 2001; Wethington & Guralnick, 2004; Wethington et al., in prep.) and microsatellite polymorphisms (Bousset et al., 2004). Controlled breeding studies have uncovered little reproductive isolation among physid populations long assumed to represent different species, prompting calls for a reappraisal of systematic relationships within the family (Dillon et al., 2002, 2004; Dillon & Wethington, 2004; Dillon et al., in press 2). The classification system proposed by Wethington (2003; Wethington & Lydeard, in press) would return the number of genera to two – *Physa* and *Aplexa*.

Ideally, a new classification of the Physidae would integrate Te's morphological observations with more recent allozyme, DNA, and breeding data into a single unified system. Unfortunately, however, Te did not report collection localities or museum lot numbers for the 85 taxa upon which his 1978 classification was based, nor did he provide figures, keys, or any practical method by which the species

¹Department of Biology, College of Charleston, Charleston, South Carolina 29424, U.S.A.; dillonr@cofc.edu

²Science Department, Chowan College, 200 Jones Drive, Murfreesboro, North Carolina 27855, U.S.A.

he recognized might be distinguished. Since any effort to modernize or update Te's system would ideally begin with a resampling of his taxa to gather correlative genetic information, progress in physid systematics has been slowed.

Fortunately, Te (1975) did publish one preliminary paper, "Michigan Physidae, with systematic notes on *Physella* and *Physodon*". Although limited to just the six species and eight subspecies he recognized in the state, Te provided figures, a dichotomous key (based on shell characters), anatomical notes, synonymy, range data, and a "partial phylogenetic tree" for this subset. The purpose of the present paper is to report the results of a survey of genetic divergence at allozyme-encoding loci among a large sample of physid populations from Michigan, identified using the conchological key of Te (1975), as a step toward reconciling Te's 1978 classification with more recent classifications based on genetic data (Wethington, 2003; Wethington & Lydeard, in press).

The physid fauna of Michigan includes three nominal species sharing the "type B" penial morphology, *Physa gyrina*, *P. sayii*, and *P. parkeri*, all assigned by Te to the subgenus "*Physella*". He noted some minor differences among these three species in the length ratios of the glandular and non-glandular portions of their penial sheaths, as well as the transparency of the non-glandular region and terminal swelling in the glandular. But Te (1975) wrote, "*Physa gyrina*, *P. sayii* and *P. parkeri* are all related in one species complex. As such, there are intermediate forms that may be difficult to place; this is especially a problem between *P. gyrina* and *P. sayii*."

Burch & Jung (1992) also found the Michigan species of the subgenus *Physella* difficult to distinguish. They wrote, "Our approach has been to note morphological groups that correspond to named entities (nominal species) that seem distinct enough to possibly be good species." Burch & Jung recognized four "named entities" of *Physella* (s.s.) inhabiting northern Michigan: globose, strongly shouldered *P. parkeri*, elliptical or elongate-ovate *P. gyrina*, ovate thin *P. sayii*, and ovate thick *P. magnalacustris*, which Te considered a subspecies of *P. sayii*. As the systematic relationships within this group have continued to prove especially problematic, populations of physids from the subgenus *Physella* were the objects of particular attention in the investigation reported here.

METHODS

Our field survey was designed to sample the physid species reported by Te (1975), identified using the conchological key he provided, collected from their representative ranges across the state of Michigan. Ultimately, we sampled 16 populations, including two of *Aplexa hypnorum*, two of *Physa sayii*, three of *Physa parkeri*, four of *Physa gyrina*, and five of *Physa acuta*. The last-listed species was identified as "*P. integra*" by Te, a name that has subsequently been synonymized (Dillon et al., 2002). Sample sites are shown in Figure 1, with locality data and sample sizes listed in the Appendix. We were unable to collect the sixth species reported by Te, *Physa jennessi*, from any of the seven Michigan sites he listed.

Whole-snail homogenates were centrifuged and analyzed via horizontal starch gel electrophoresis using methods and apparatus as described by Dillon (1992). Multiple buffer systems were employed where possible to screen for hidden variation (Coyne & Felton, 1978). The AP6 buffer system of Clayton & Tretiak



FIG. 1. Outline map of the state of Michigan, showing sample sites. A = *Aplexa hypnorum*, G = *Physa gyrina*, I = *Physa acuta*, P = *Physa parkeri*, S = *Physa sayii*. See Appendix for locality data.

(1972) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), leucine aminopeptidase (LAP), glucose phosphate isomerase (GPI), and isocitrate dehydrogenase (ISDH). We employed the TC6.8 buffer system of Mulvey & Vrijenhoek (1981) to resolve GPI, ISDH, phosphoglucosmutase (PGM2), and mannose phosphate isomerase (MPI). The TEB8 system (buffer III of Shaw & Prasad, 1970) was used to analyze LAP, 6PGD, and the esterases (EST3).

Our initial runs included control samples of the well-characterized *P. acuta* population inhabiting the main pond at Charles Towne Landing State Park, Charleston, South Carolina (population C or CTL in Dillon & Wethington, 1995; Dillon et al., 2002; Wethington & Dillon, 1991). Putative alleles were named according to the electrophoretic mobility of their allozyme products in millimeters, setting the mobility of the most common allele in population C to 100. Mendelian interpretation has

been confirmed for EST3 and LAP by Dillon & Wethington (1994), and for GPI, PGM, and 6PGD in planorbids by Mulvey & Vrijenhoek (1984) and Mulvey et al. (1988).

Data analysis was performed using Biosys version 1.7 (Swofford & Selander, 1981). Because large numbers of alleles were resolved at some loci, our sample sizes dictated that genotypes be pooled into three classes: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes before testing for Hardy-Weinberg equilibrium. Yates-corrected chi-square statistics were then employed for this purpose. We calculated matrices of Nei (1978) unbiased genetic identity and Cavalli-Sforza & Edwards (1967) chord distance. As distances of the latter type are Pythagorean in Euclidean space, they were used as the basis for an UPGMA cluster analysis (Wright, 1978) and a neighbor-joining tree (PAUP* 4.0b10; Swofford 1998).

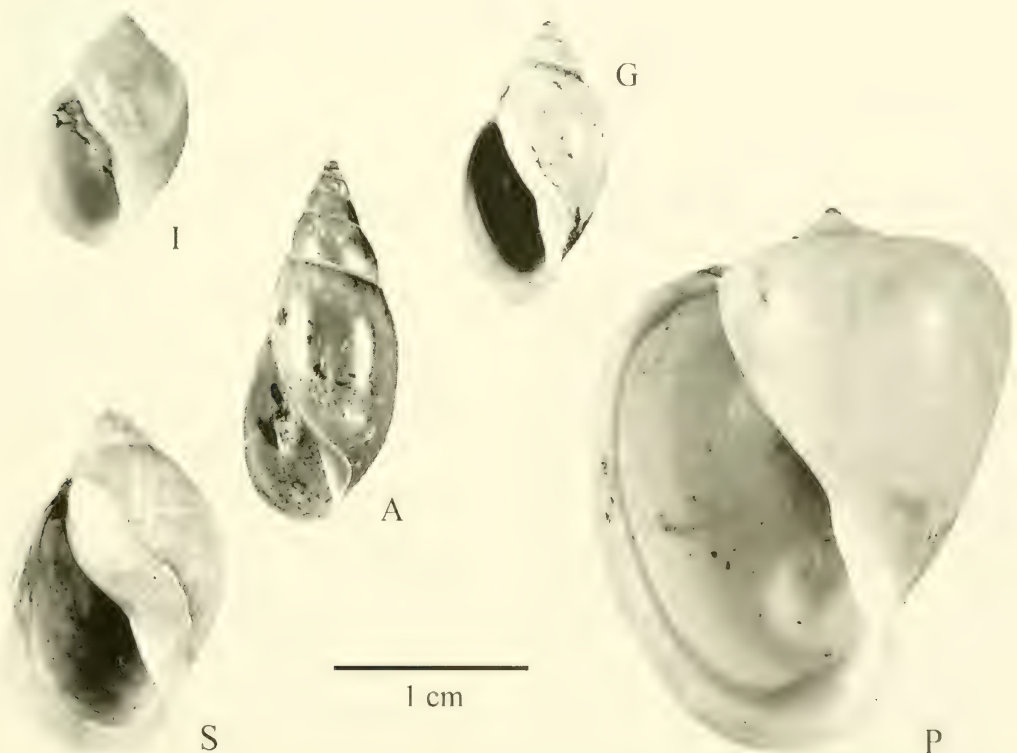


FIG. 2. Exemplar shells of the five physid species examined in this study. I – *Physa acuta* (population I1), S – *Physa sayii* (population S1), G – *Physa gyrina* (population G1), A – *Aplexa hypnorum* (population A2), P – *Physa parkeri* (population P1). See appendix for locality data.

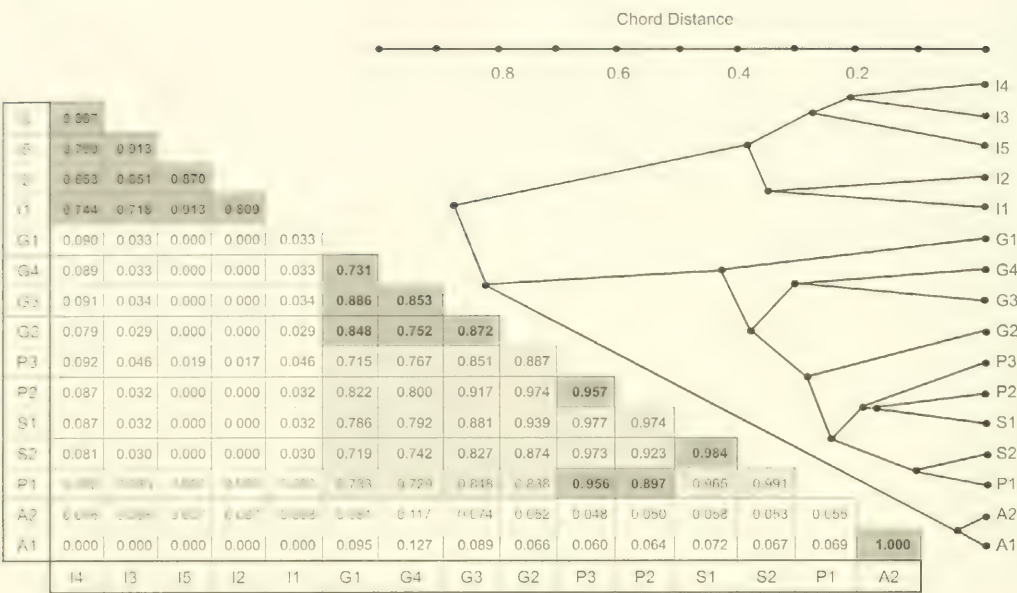


FIG. 3. Nei's (1978) unbiased genetic identities are shown below the diagonal, with nominally conspecific comparisons darkly shaded and other comparisons within the *gyrina* complex shaded lightly. Above the diagonal is the result of a UPGMA cluster analysis based on Cavalli-Sforza & Edwards (1967) chord distance.

RESULTS

We found Te's (1975) conchological key difficult to apply to natural populations collected from the wild, failing entirely in smaller individuals. Although *Aplexa* and (generally) *P. acuta* could be distinguished unambiguously, shell morphological variation within and among populations of *P. gyrina*, *P. sayii*, and *P. parkeri* often thwarted positive identification. Nor have any anatomical distinctions been subsequently described that might facilitate this process. We would have preferred to sample more populations of *P. sayii* in particular, but intergradation with both *P. gyrina* and *P. parkeri* made identification of this taxon especially problematic. The shells chosen for illustration in Figure 2 are exemplars. Voucher specimens have been deposited in the University of Michigan Museum of Zoology.

Allele frequencies at the seven enzyme-encoding loci are given in Table 1. Of the 16 x 7 = 112 loci examined, a total of 54 were polymorphic by the 95% criterion. Chi-square analysis revealed heterozygote deficits nominally significant at the 0.05 level in six of these cases – Est3 at population I4, Isdh in population I3, Est3 in population G3, and three polymorphic loci in population I5: Est3, Lap, and Isdh.

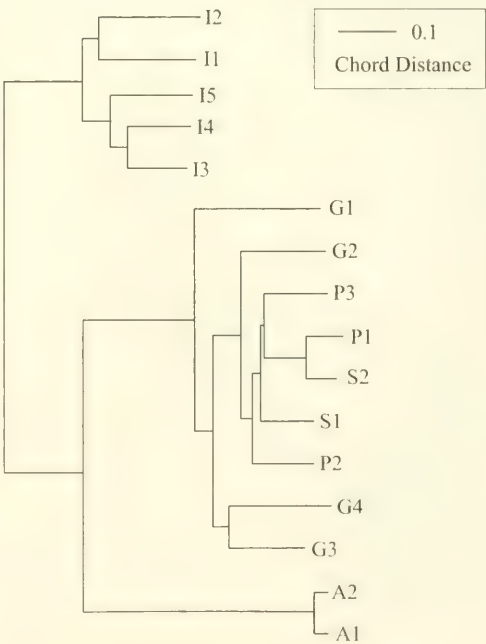


FIG. 4. Neighbor-joining tree (PAUP*; Swofford 1998) based on the matrix of Cavalli-Sforza & Edwards (1967) chord distance.

TABLE 1. Gene frequencies at seven polymorphic enzyme loci in 16 populations of physid snails from Michigan.

Allele	A1	A2	G1	G2	G3	G4	I1	I2	I3	I4	I5	P1	P2	P3	S1	S2
EST3																
104	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.411	0.136	0.379	0.000	0.000	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.563	0.864	0.345	0.000	0.000	0.000	0.000	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.839	0.933	0.027	0.000	0.276	0.000	0.000	0.000	0.000	0.000
91	0.000	0.000	0.000	0.018	0.152	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.000	0.000	0.081	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
89	0.000	0.000	0.000	0.982	0.217	0.741	0.000	0.000	0.000	0.000	0.000	0.611	0.857	0.859	0.849	0.871
87	0.000	0.000	0.379	0.000	0.630	0.259	0.000	0.000	0.000	0.000	0.000	0.343	0.125	0.141	0.023	0.129
84	0.000	0.000	0.621	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.047	0.000
LAP																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.813	0.288	0.264	0.672	0.192	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.712	0.698	0.328	0.808	0.000	0.000	0.000	0.000	0.000
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.000	0.850	1.000	0.771	0.679	0.000	0.000	0.000	0.000	0.000	0.990	0.958	0.926	0.938	1.000
88	0.000	0.000	0.150	0.000	0.229	0.321	0.000	0.000	0.000	0.000	0.000	0.010	0.042	0.074	0.063	0.000
82	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PGD																
100	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.964	0.750	0.942	0.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.250	0.019	0.000	0.000	0.000	0.000	0.000
94	0.325	0.348	0.000	0.000	0.000	0.107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.013
92	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.175	0.130	0.643	1.000	0.783	0.000	0.000	0.000	0.000	0.000	0.000	0.990	1.000	0.968	0.938	0.987
86	0.500	0.478	0.357	0.000	0.217	0.893	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.032	0.013	0.000

(continues)

Figure 3 shows the matrix of Nei's genetic identity among all pairs of populations and the results of an UPGMA cluster analysis based on Cavalli-Sforza and Edwards Chord distance. The cophenetic correlation (Sokal & Rohlf, 1962) for this analysis was very high, $r_{CS} = 0.993$ (Sneath & Sokal, 1973: 304), indicating a good fit between the branch length and the original distance matrix. The neighbor-joining tree is shown in Figure 4.

DISCUSSION

Fits to Hardy-Weinberg expectation were good in almost all populations, with scattered nominally significant values of chi square probably attributable to Type 1 statistical error. The exception was population I5, where significant heterozygote deficits were apparent at three of five polymorphic loci examined. Outcrossing is strongly preferred in laboratory populations of *Physa acuta*, self-fertilization resulting in a substantial fitness decrement (Wethington & Dillon, 1993, 1996, 1997). Evidence of inbreeding has nevertheless often been reported in natural populations of *Physa* (Dillon & Wethington, 1995; Jarne et al., 2000) and other pulmonates (Jarne 1995). Some low level of self-fertilization may be an unavoidable consequence of the pulmonate reproductive system (Dillon et al., in press 1). At the I5 site, low population densities may have increased the frequency of self-fertilization beyond the background levels that were more difficult to detect in other populations at our sample sizes.

Both the neighbor-joining tree and the UPGMA cluster analysis revealed three distinct groups – the two populations of *Aplexa* together, the five populations of *P. acuta* together, and the nine populations of *P. gyrina*, *P. sayii*, and *P. parkeri* combined (Figs. 3, 4). The five *P. acuta* populations, clustered at a chord distance of 0.37, showed a minimum genetic identity of 0.718. This is quite similar to the level of genetic divergence among the ten populations of *P. acuta* sampled from the Charleston area by Dillon & Wethington (1995). This level is also strikingly similar to that displayed within the nine populations of the *gyrina/sayii/parkeri* group, clustered at a chord distance of 0.43 with a minimum genetic identity of 0.715. The specific distinction between *P. gyrina*, *P. sayii*, and *P. parkeri*, hereafter referred to as the “*gyrina* group”, is called into question.

Physa gyrina ranges broadly across North America, throughout Canada and the United States as far south as Virginia and Kentucky. In Michigan, Te reported populations from a wide variety of shallow habitats – creeks, brooks, pools, ponds, and ditches. The ranges of *Physa sayii* and *P. parkeri* are more restricted to the Great Lakes region and to deeper waters, Te giving the habitat of the former as “lakes and rivers” and the habitat of the latter as “large lakes”.

Both Figures 3 and 4 depict the *sayii/parkeri* cluster as a subset within the larger *gyrina* group. This suggests to us that the generally larger, inflated, and globose shell that characterizes populations referred to these two nomina may be a regional (and possibly ecophenotypic) response to the colonization of lacustrine habitats by populations of the more typical *P. gyrina* morphology. We hypothesize that individuals inhabiting larger lakes and rivers may tend to live longer, and hence grow larger of body, than individuals inhabiting ponds and creeks. It also possible that the rotund, globose and often shouldered shell phenotype characterizing *P. parkeri* (and sometimes *P. sayii*) may be related to a deepwater habitat unaffected by current or wind.

The tendency for physid snails to develop rotund shells as a phenotypically plastic response to the threat of fish predation is well documented (DeWitt, 1998; DeWitt et al., 1999, 2000; Langerhans & DeWitt, 2002). More recently, Britton & McMahon (2004) have reported that physids respond to increased water temperature by developing wider shell spire angle, a variable positively correlated with shell globosity. It seems clear that the minor differences in shell morphology upon which rest the distinctions among the several nominal species of the *gyrina* group need not reflect any heritable variance whatsoever.

Breeding experiments would provide the ideal test to confirm that the three nominal species of the *gyrina* group inhabiting Michigan are in fact biologically conspecific. Dillon & Wethington (2004) reported the results of no-choice mating experiments between a line of *P. parkeri* from Douglas Lake and *P. gyrina* collected from its type locality near Council Bluffs, Iowa. Our control *P. parkeri* hatched and reared under laboratory conditions did not develop the shoulder on their shell characteristic of wild-collected animals, remaining superficially indistinguishable from control *P. gyrina*. Control *parkeri* hybridized readily with

P. gyrina, producing viable F1 offspring. The growth, survival rate, and fecundity of *P. parkeri* were, however, significantly below those posted by control *P. gyrina*, in both the control pairs and in the outcross *parkeri* x *gyrina* experiment. We were ultimately unable to carry either control *P. parkeri* or *parkeri* x *gyrina* hybrids to the F2 generation under our culture conditions, leaving the question of reproductive isolation an open one. Our experiments nevertheless confirmed that the life history adaptations evolved by *P. parkeri* have a heritable basis, although some key aspects of shell morphology, upon which the taxonomy is based, may not.

The overall form of the analyses shown in Figures 3 and 4 is consistent with the phylogeny suggested by Wethington (2003) and Wethington & Lydeard (in press). Mitochondrial COI and 16s sequence data, analyzed via parsimony, yielded a tree in which the genera *Aplexa* and *Physa* split first, followed by a split between the clade containing *P. acuta* and the clade containing the *gyrina* group. The analysis of Wethington & Lydeard also resolved two clades within the *gyrina* group: a "typical" subset and a "globose" subset that included *parkeri* and *sayii* (subspecies *magnalacustris*.) The authors attributed this distinction to geographical factors, however, not to reproductive isolation.

Our allozyme data, taken together with the partial results of the Dillon & Wethington (2004) breeding experiments, suggest that the nominal taxa *P. parkeri* and *P. sayii* may best be treated as junior synonyms of *P. gyrina*. Final confirmation of this hypothesis will await careful analysis of reproductive interactions between populations of these three nominal species in natural sympatry. Given the difficulty we and other workers have encountered distinguishing members of the *gyrina* group in the field, however, it may materialize that no practical site for such a study can be identified.

The 85 taxonomic units upon which Te (1978, 1980) based his classification included all 14 of the taxa he recognized from Michigan: *Aplexa hypnorum* (*tryoni* and *hypnorum* s.s.), *Physa jennessi* (subspecies *skinneri*), *Physa gyrina* (*elliptica*, *hildrethiana*, and *gyrina* s.s.), *Physa sayii* (*magnalacustris*, *vinosa*, and *sayii* s.s.), *Physa parkeri* (*latchfordii* and *parkeri* s.s.), and *Physa integra* (*brevispira*, *walkerii*, and *integra* s.s.). Including *P. jennessi*, the validity of which we have no reason to doubt, our allozyme data suggest that these 14 taxa may comprise just four biological species. It is

clear that Te's analysis was based on a set of taxonomic units divided much more finely than biological species. This suggests to us that the revised classification of Wethington & Lydeard, returning the Physidae to a simpler two-genus system, has much to recommend it.

ACKNOWLEDGEMENTS

Field assistance was provided by Joseph Reznick, Zeldia Wethington, Jennifer Walker, Jennifer Stephens, and Eileen Jokinen. J. B. Burch provided some insight regarding the identifications. This research was supported by a grant from the National Science Foundation, DEB-0128964, and by a travel grant from the graduate student council at the University of Alabama.

LITERATURE CITED

- BOUSSET, L., P.-Y. HENRY, P. SOURROUILLE & P. JARNE, 2004, Population biology of the invasive freshwater snail *Physa acuta* approached through genetic markers, ecological characterization and demography. *Molecular Ecology*, 13: 2023–2036.
- BRITTON, D. K. & R. F. MCMAHON, 2004, Environmentally and genetically induced shell-shape variation in the freshwater pond snail *Physa (Physella) virgata* (Gould, 1855). *American Malacological Bulletin*, 19: 93–100.
- BROWN, K. M., 1991, Gastropoda. Pp. 285–314, in: J. H. THORP & A. P. COVICH, eds., *Ecology and classification of North American freshwater invertebrates*. Academic Press, New York. 911 pp.
- BURCH, J. B., 1989, *North American freshwater snails*. Malacological Publications, Hamburg, Michigan. 365 pp.
- BURCH, J. B. & Y. JUNG, 1992, Freshwater snails of the University of Michigan Biological Station area. *Walkerana*, 6: 1–218.
- BUTH, D. G. & J. J. SULOWAY, 1983, Biochemical genetics of the snail genus *Physa*: a comparison of populations of two species. *Malacologia*, 23: 351–359.
- CAVALLI-SFORZA, L. L. & A. W. F. EDWARDS, 1967, Phylogenetic analysis: models and estimation procedures. *Evolution*, 21: 550–570.
- CLAYTON, J. W. & D. N. TRETIAK, 1972, Amine-citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada*, 29: 1169–1172.
- COYNE, J. A. & A. A. FELTON, 1978, Genic heterogeneity at two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *Drosophila persimilis*. *Genetics*, 87: 285–304.
- DEWITT, T. J., 1998, Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *Journal of Evolutionary Biology*, 11: 465–480.

- DEWITT, T. J., B. W. ROBINSON & D. S. WILSON, 2000, Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evolutionary Ecology Research*, 2: 129–148.
- DEWITT, T. J., A. SIH & J. A. HUCKO, 1999, Trait compensation and cospecialization in a freshwater snail: size, shape, and antipredator behaviour. *Animal Behaviour*, 58: 397–407.
- DILLON, R. T., JR., 1992, Electrophoresis IV, nuts and bolts. *World Aquaculture*, 23(2): 48–51.
- DILLON, R. T., JR., 2000, *The ecology of freshwater molluscs*. Cambridge University Press, Cambridge. 509 pp.
- DILLON, R. T., JR., C. E. EARNHARDT & T. P. SMITH, 2004, Reproductive isolation between *Physa acuta* and *Physa gyrina* in joint culture. *American Malacological Bulletin*, 19: 63–68.
- DILLON, R. T., JR., T. E. MCCULLOUGH & C. E. EARNHARDT, in press 1, Estimates of natural allosperm storage capacity and self-fertilization rate in the hermaphroditic freshwater pulmonate snail, *Physa acuta*. *Invertebrate Reproduction and Development*.
- DILLON, R. T., JR., J. D. ROBINSON, T. P. SMITH & A. R. WETHINGTON, in press 2, No reproductive isolation between the freshwater pulmonate snails *Physa acuta* and *Physa virgata*. *Southwestern Naturalist*.
- DILLON, R. T., JR. & A. R. WETHINGTON, 1994, Inheritance at five loci in the freshwater snail, *Physa heterostropha*. *Biochemical Genetics*, 32: 75–82.
- DILLON, R. T., JR. & A. R. WETHINGTON, 1995, The biogeography of sea islands: clues from the population genetics of the freshwater snail, *Physa heterostropha*. *Systematic Biology*, 44: 400–408.
- DILLON, R. T., JR. & A. R. WETHINGTON, 2004, No-choice mating experiments among six nominal taxa of the subgenus *Physella* (Basommatophora: Physidae). *Heldia*, 6: 69–78.
- DILLON, R. T., JR., A. R. WETHINGTON, J. M. RHETT & T. P. SMITH, 2002, Populations of the European freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. *Invertebrate Biology*, 121: 226–234.
- JARNE, P., 1995, Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals. *Genetical Research*, 65: 193–207.
- JARNE, P., M.-A. PERDIEU, A.-F. PERNOT, B. DELAY & P. DAVID, 2000, The influence of self-fertilization and grouping on fitness attributes in the freshwater snail *Physa acuta*: population and individual inbreeding depression. *Journal of Evolutionary Biology*, 13: 645–655.
- LANGERHANS, R. B. & T. J. DEWITT, 2002, Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. *Evolutionary Ecology Research*, 4: 857–870.
- LIU, H.-P., 1993, Diagnostic genetic loci for species in the genus *Physella*. *Malacological Review*, 26: 1–8.
- MULVEY, M. & R. C. VRIJENHOEK, 1981, Genetic variation among laboratory strains of the planorbid snail *Biomphalaria glabrata*. *Biochemical Genetics*, 19: 1169–1182.
- MULVEY, M. & R. C. VRIJENHOEK, 1984, Genetics of *Biomphalaria glabrata*: linkage analysis and crossing compatibilities among laboratory strains. *Malacologia*, 25: 513–524.
- MULVEY, M., D. S. WOODRUFF & M. P. CARPENTER, 1988, Linkage relationships of seven enzyme and two pigmentation loci in the snail *Biomphalaria glabrata*. *Journal of Heredity*, 79: 473–476.
- NEI, M., 1978, Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583–590.
- REMIGIO, E. A., D. A. W. LEPITZKI, J. S. LEE & P. D. N. HEBERT, 2001, Molecular systematic relationships and evidence for a recent origin of the thermal spring endemic snails *Physella johnsoni* and *Physella wrighti* (Pulmonata: Physidae). *Canadian Journal of Zoology*, 79: 1941–1950.
- SHAW, C. R. & R. PRASAD, 1970, Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochemical Genetics*, 4: 297–320.
- SNEATH, P. H. A. & R. R. SOKAL, 1973, *Numerical taxonomy*. W. H. Freeman, San Francisco. 573 pp.
- SOKAL, R. R. & F. J. ROHLF, 1962, The comparison of dendrograms by objective methods. *Taxon*, 11: 33–40.
- SWOFFORD, D. L., 1998, *PAUP* phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.
- SWOFFORD, D. L. & R. B. SELANDER, 1981, BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity*, 72: 281–283.
- TE, G. A., 1975, Michigan Physidae, with systematic notes on *Physella* and *Physodon* (Basommatophora: Pulmonata). *Malacological Review*, 8: 7–30.
- TE, G. A., 1978, The systematics of the family Physidae (Basommatophora: Pulmonata). Dissertation, University of Michigan, Ann Arbor, Michigan. 325 pp.
- TE, G. A., 1980, New classification system for the family Physidae. *Archiv für Molluskenkunde*, 110: 179–184.
- TURGEON, D. D., J. F. QUINN, A. E. BOGAN, E. V. COAN, F. G. HOCHBERG, W. G. LYONS, P. M. MIKKELSEN, R. J. NEVES, C. F. E. ROPER, G. ROSENBERG, B. ROTH, A. SCHELTEMA, F. G. THOMPSON, M. VECCHIONE & J. D. WILLIAMS, 1998, *Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks*. Special Publications, Vol. 26. American Fisheries Society, Bethesda, Maryland. 526 pp.
- WETHINGTON, A. R., 2003, Phylogeny, taxonomy, and evolution of reproductive isolation in *Physa* (Pulmonata: Physidae). Dissertation, University of Alabama, Tuscaloosa, Alabama. 119 pp.
- WETHINGTON, A. R. & R. T. DILLON, JR., 1991, Sperm storage and evidence for multiple in-

- semination in a natural population of the freshwater snail, *Physa*. *American Malacological Bulletin*, 9: 99–102.
- WETHINGTON, A. R. & R. T. DILLON, JR., 1993, Reproductive development in the hermaphroditic freshwater snail, *Physa*, monitored with complementing albino lines. *Proceedings of the Royal Society of London (B)*, 252: 109–114.
- WETHINGTON, A. R. & R. T. DILLON, JR., 1996, Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*. *Animal Behaviour*, 51: 1107–1118.
- WETHINGTON, A. R. & R. T. DILLON, JR., 1997, Selfing, outcrossing, and mixed mating in the freshwater snail *Physa heterostrophia*: lifetime fitness and inbreeding depression. *Invertebrate Biology*, 116: 192–199.
- WETHINGTON, A. R. & R. GURALNICK, 2004, Are populations of physids from different hot springs distinctive lineages? *American Malacological Bulletin*, 19: 135–144.
- WETHINGTON, A. R. & C. LYDEARD, in press, A molecular phylogeny of Physidae (Gastropoda: Basommatophora) based on mitochondrial DNA sequences. *Journal of Molluscan Studies*.
- WETHINGTON, A. R., J. M. RHETT & R. T. DILLON, JR., in prep., Allozyme, 16S, and CO1 sequence divergence among six populations of the cosmopolitan freshwater snail, *Physa acuta*.
- WRIGHT, S., 1978, *Variability within and among natural populations*. Vol. 4, *Evolution and the genetics of populations*. University of Chicago Press, Chicago, Illinois. 580 pp.
- Marquette Co., Michigan. 46.2815°N, 87.3337°W. N = 31.
- G2 *Physa gyrina*. Little Carp River at Hogsback Rd., 1 km N of Burt Lake, Cheboygan Co., Michigan. 45.5520°N, 84.6854°W. N = 28.
- G3 *Physa gyrina*. Turtle Lake at Miller Rd., 5 km W of Bendon, Benzie Co., Michigan. 44.6178°N, 85.9090°W. N = 24.
- G4 *Physa gyrina*. Twin Sun Lakes at Highgate Beach, Wixom, Oakland Co., Michigan. 42.5466°N, 83.5085°W. N = 33.
- I1 *Physa acuta*. Douglas Lake at the University of Michigan Biological Station, Cheboygan Co., Michigan. 45.5634°N, 84.6783°W. N = 32.
- I2 *Physa acuta*. Higgins Lake near boat ramp at Sam O Set Blvd., Sharps Corners, Roscommon Co., Michigan. 44.4246°N, 84.6942°W. N = 31.
- I3 *Physa acuta*. Saginaw Bay at Quanicasee Wildlife Area, Tuscola Co., Michigan. 43.5896°N, 83.6774°W. N = 57.
- I4 *Physa acuta*. Pond near the junction of Mi 11 and Mi 37, Grand Rapids, Kent Co., Michigan. 42.9168°N, 85.5771°W. N = 44.
- I5 *Physa acuta*. Kent Lake at Kensington MetroPark, Oakland Co., Michigan. 42.5336°N, 83.6462°W. N = 29.
- P1 *Physa parkeri*. Douglas Lake at the University of Michigan Biological Station, Cheboygan Co., Michigan. 45.5634°N, 84.6783°W. N = 59.
- P2 *Physa parkeri*. Long Lake at Long Lake Rd., 10 km SE of Traverse City, Grand Traverse Co., Michigan. 44.7140°N, 85.7316°W. N = 37.
- P3 *Physa parkeri*. Higgins Lake near boat ramp at Sam O Set Blvd., Sharps Corners, Roscommon Co., Michigan. 44.4246°N, 84.6942°W. N = 47.
- S1 *Physa sayii*. Lake Michigan at Wilderness State Park, Emmet Co., Michigan. 45.7474°N, 84.9045°W. N = 49.
- S2 *Physa sayii*. Crystal Lake 3 km N of Frankfort, Benzie Co., Michigan. 44.6607°N, 86.2320°W. N = 39.

Revised ms. accepted 1 September 2005

APPENDIX

Locality data and sample sizes

- A1 *Aplexa hypnorum*. Woodland pond at the Maple Bay access of Burt Lake, Cheboygan Co., Michigan. 45.4867°N, 84.7088°W. N = 21.
- A2 *Aplexa hypnorum*. Houghton Lake at state campground, Roscommon Co., Michigan. 44.3388°N, 84.6648°W. N = 26.
- G1 *Physa gyrina*. Little Lake at state campground, 1 km S of town of Little Lake,